• In re Application of:

Pan et al.

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PATENT Attorney Docket No.: AERO1210-2

Amendment to the Specification:

Please replace paragraph [0080] with the following:

[0080] An additional aspect of this invention is provided in the method with which the protein is expressed and refolded as depicted in Example 2. The IL-4 mutein must be purified properly to allow efficient PEGylation. Purification is described, for example, in Example 2 below. When the mutein is refolded in the presence of a sulfhydryl protecting agent such as beta-mercaptoethanol, glutathione, or cysteine, the purified mutein can not be PEGylated because the active sulhydryl in the introduced cysteine on IL-4 is inactivated by the oxidized protecting agent. A covalent disulfide bond is formed between the IL-4 mutein's free cysteine and the protecting agent. In contrast, the use of the sulfhydryl protecting agent dithiothreitol (DTT), which oxidizes to form a stable disulfide bond, will not form a covalent bond with the IL-4 mutein's free cysteine, thus leaving its sulfydryl group free to react with the PEG maleimide reagent. IL-4 muteins purified after refolding in the presence of beta-mercaptoethanol, glutathione, or cysteine can react with the PEG reagent if treated with DTT, but a mixture of monoPEGylated and multiPEGylated products are generated, suggesting that existing IL-4 cysteines are also PEGylated. PEGylation of existing cysteines would lead to misfolded products that are inactive.